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Membrane Filtration Is Suitable for Reliable Elimination of *Mycobacterium tuberculosis* from Saliva for Therapeutic Drug Monitoring

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Tuberculosis (TB) remains an infectious disease of worldwide concern. Therapeutic drug monitoring (TDM) of blood could be helpful in optimizing TB treatment, as anti-TB drug exposure shows interpatient variability (1). TDM in saliva instead of blood is currently being studied as a more practical alternative, since saliva sampling is noninvasive and more acceptable to patients (2, 3). Along with the growing interest in the pharmacokinetics of anti-TB drugs, TDM is increasingly used in daily routine practice. However, the saliva of infectious TB patients contains *Mycobacterium tuberculosis* and TDM sample analysis usually does not take place in a biosafety level 3 laboratory. A quantitative study found a mean bacterial load of 7×10^4 (range, 1×10^2 to 6×10^5) CFU/ml in the saliva of infectious TB patients (4). Laboratory-acquired TB infections should be prevented by applying biosafety measures when working with *M. tuberculosis*-containing saliva samples (5). Therefore, saliva samples from TB patients require sterilization prior to laboratory processing (e.g., centrifugation). Unfortunately, heat sterilization is not possible because of the thermal instability of drugs. The objective of this experiment was to test whether membrane filtration is able to reliably decontaminate a solution containing *M. tuberculosis*.

Five *M. tuberculosis* strains (Table 1) were incubated in Mycobacteria Growth Indicator Tubes (MGITs; Becton, Dickinson and Company, United States) after the addition of 0.8 ml of oleic acid, albumin, dextrose, and catalase as a growth supplement. For each strain, 2.0 ml of culture fluid containing at least 10^5 to 10^6 CFU/ml was filtered in duplicate with a polyvinylidene fluoride membrane filter with a pore size of 0.22 μm and a diameter of 33 mm (Millex-GV; Merck Millipore, Ireland). The filtrate was inoculated into a new MGIT with culture fluid. For each strain, 0.5 ml of culture fluid containing at least 10^5 to 10^6 CFU/ml was also inoculated into a new MGIT as a positive control. All tubes were incubated at 36.5°C for 55 days in the Bactec MGIT 960 system (Becton, Dickinson and Company, United States). No mycobacterial growth was observed in the MGITs inoculated with filtrate, while all of the control tubes were positive within 2 weeks (Table 1).

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TABLE 1 Growth of five strains of *M. tuberculosis* in positive-control samples and filtrates[a]

Strain	Species	Drug resistance(s)	No. of growth units		
			Positive control	Filtrate A	Filtrate B
1	*M. tuberculosis* complex	Sensitive	7,037	0	0
2	*M. tuberculosis*	Isoniazid, rifampin	18,216	0	0
3	*M. tuberculosis*	Rifampin	20,413	0	0
4	*M. tuberculosis*	Sensitive	26,757	0	0
H37Rv	*M. tuberculosis*	Sensitive	22,776	0	0

[a]In duplicate (A and B).

This is the first description of membrane filtration of *M. tuberculosis*-containing fluids for sterilization in the process of TDM. No mycobacterial growth was measured in any of the filtrates. The membrane filter therefore successfully filtered all of the bacteria of multiple *M. tuberculosis* strains from culture fluids. We found no difference among the five strains in the number of growth units in the filtrates. It is not possible to test all of the *M. tuberculosis* isolates received at a mycobacterial laboratory, but according to this experiment, variation in the feasibility of membrane filtration between different strains is not likely. Membrane filtration of solutions with a larger bacterial load than that tested here requires further investigation, as sterilization cannot be ensured by only this experiment. However, the bacterial load in saliva from TB patients is usually not as large as that tested in this experiment (4). Because of the satisfying results obtained with culture fluids with large bacterial loads, we conclude that membrane filtration is suitable for the decontamination of salivary TDM samples from infectious TB patients.

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